(11)1 556 853

PATENT SPECIFICATION

(21) Application No. 19981/78

(22) Filed 16 May 1978

(31) Convention Application No. 2742874 (32) Filed 23 Sep. 1977 in

(33) Fed. Rep. of Germany (DE)

(44) Complete Specification Published 28 Nov. 1979

B01D 53/14 53/16 (51) INT. CL.² C12B 1/00

(52) Index at Acceptance C6F CA 110 116 13 D8 G50 G50D8 VG6 ClA



(54) OXYGEN-ABSORBING AGENTS

(71) We. MERCK PATENT GESELL-SCHAFT MIT BESCHRANKTER HAF-TUNG, of 250, Frankfurter Strasse, 61 Darmstadt, Federal Republic of Germany, a Joint-Stock Company organised under the laws of the Federal Republic of Germany. do hereby declare the invention, for which we pray that a patent may be granted to us. and the method by which it is to be performed, to be particularly described in and by the following statement:-

The present invention is concerned with oxygen-absorbing agents and with a process for the production of an oxygen-poor or oxygen-free atmosphere for culturing anaerobic bacteria with the use of these

oxygen-absorbing agents.

Oxygen-absorbing agents have long been used for the reduction or removal of atmospheric oxygen for anaerobic culture processes. Thus, for example, use has been made of mixtures of pyrogallol powder, sodium carbonate and kieselguhr, which are thoroughly moistened either by adding water or by water vapour from the nutrient substrate and then absorb oxygen. J. Clin. Microbiol.. 1975, page 527 also described a mixture of steel wool and acidified copper sulphate solution for this purpose.

The known mixtures have the disadvantage that their absorption ability for oxygen is not optimal. In the case of the abovementioned mixture described in J. Clin. Microbiol., an acidic copper sulphate solution must first be prepared with which the steel wool is then saturated. After some time, the excess of this solution must be discarded.

It is an object of the present invention to provide an oxygen-absorbing agent which possesses an optimum absorption ability, is simple to handle and can be used immedi-

ately.

Thus, according to the present invention. there is provided an oxygen-absorbing agent, comprising an adsorption agent, metal powder or turnings and an activator.

The present invention also provides a process for the production of an oxygenpoor or oxygen-free atmosphere for culturing anaerobic bacteria, wherein an oxygenabsorbing agent according to the present invention is brought into contact with a reaction mediator.

Surprisingly, we have found that the agent according to the present invention, in comparison with the mixture described in J. Clin. Microbiol., possesses a 5 to 10 times better oxygen absorption ability, referred to the amount of iron.

The adsorption agent contained in the agent according to the present invention serves to suck up the liquid reaction mediator and thus to prevent a deliquescence of the mixture. Examples of adsorption agents which can be used include kieselguhr, silica gel and cellulose, kieselguhr being preferred.

Examples of metal powders or turnings which can be used include those of heavy metals, such as iron, manganese, cobalt, nickel and the like, iron powder being preferred.

Examples of activators which can be used include organic acids, such as citric acid, tartaric acid, dilute acetic acid and the like, dilute mineral acids, such as hydrochloric acid and sulphuric acid, as well as complexing agents and salts of noble metals. A preferred activator according to the present invention is citric acid.

In many cases, it is desirable to add sodium carbonate to the oxygen-absorbing agent in order, for example, to provide in the closed culture vessel a certain pressure equilisation for the absorbed oxygen or because some bacteria require an increased content of carbon dioxide in the atmosphere for optimum growth.

The weight ratio of the individual mixture 90

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	components adsorption agent; metal pow-	Example 2	
	der : activator : sodium carbonate is prefer-	Analogously to Example 1, aliquot parts	
	ably about 8:4:3:1. In the case of mixtures	of a mixture consisting of	
	without carbon dioxide evolution, i.e. with-	5.0 kg. kieselguhr,	60
5	out the addition of sodium carbonate, this	2.5 kg. cobalt powder,	
	weight ratio is preferably from about 8:4:2	1.5 kg. tartaric acid and	
	to 8:4:1. The ratio of sodium carbonate to	0.5 kg. sodium carbonate	
	citric acid activator depends essentially	are used as oxygen-absorbing agent for	
	upon the desired amount of carbon dioxide.	culturing anaerobic bacteria.	65
10	The ratio of adsorption agent to metal	Tantaring anacrosic vactoria.	w
	powder can be varied within wide limits. In	Frample 3	
	the case of kieselguhr and iron powder, the	Example 3 For the production of an example poor	
		For the production of an oxygen-poor	
	weight ratio can be from 5:1 to 1:1 and is	atmosphere without the simultaneous evolu-	70
15	preferably about 2:1. When substantially	tion of carbon dioxide, analogously to Ex-	70
15	more or less iron is employed, the oxygen-	ample 1, there are used aliquot parts of one	
	absorption ability of the agent according to	of the following mixtures:	
	the present invention decreases.	a) 5.0 kg. silica gel,	
	For the production of an oxygen-poor or	2.5 kg. iron powder and	
	oxygen-free atmosphere, such as is neces-	1.0 kg. citric acid	75
20	sary, for example, for culturing anaerobic	b) 5.0 kg. kieselguhr,	
	bacteria, the dry mixture according to the	2.5 kg. cobalt powder and	
	present invention is brought into contact	0.8 kg. tartaric acid.	
	with a reaction mediator. The reaction	WHAT WE CLAIM IS:-	
	mediator can be a solvent, for example	1. An oxygen-absorbing agent, compris-	80
25	water or ethylene glycol or a liquid acid, for	ing an adsorption agent, metal powder or	~~
	example acetic acid, dilute hydrochloric	turnings and an activator.	
	acid. sulphuric acid or the like, which, in	2. An oxygen-absorbing agent accord-	
	turn, simultaneously act as activator and	ing to claim 1, comprising kieselguhr, iron	
	reaction mediator. The preferred reaction	powder and citric acid.	85
30	mediator is water.	3. An oxygen-absorbing agent accord-	O)
50	As soon as the reaction mediator comes	ing to claim 1 or 2, which additionally	
	into contact with the oxygen-absorbing	contains sodium carbonate.	
	agent, the moist mixture begins to absorb	4. An oxygen-absorbing agent according to claim 1, substantially as hereinbefore	00
25	the oxygen comparatively quickly, with the	ing to claim 1, substantially as hereinbefore	90
35	simultaneous evolution of carbon dioxide	described and exemplified.	
	when sodium carbonate is present.	5. A process for the production of an	
	The following Examples are given for the	oxygen-poor or oxygen-free atmosphere for	
	purpose of illustrating the present inven-	culturing anaerobic bacteria, wherein an	0.5
40	tion:-	oxygen-absorbing agent according to any of	95
40	F / 1	claims 1 to 4 is brought into contact with a	
	Example 1	reaction mediator.	
	75 1. 1. 1. 1. 20 0	6. A process according to claim 5,	
	For culturing anaerobic bacteria, 30 g. of	wherein the reaction mediator is a solvent.	
	a mixture consisting of	7. A process according to claim 6,	100
45	5.0 kg. kieselguhr.	wherein the solvent is water.	
	2.5 kg. iron powder.	8. A process according to claim 5 for the	
	1.9 kg. citric acid and	production of an oxygen-poor or oxygen-	
	0.6 kg. sodium carbonate	free atmosphere, substantially as hereinbe-	
	are filled into a paper sachet and placed in	fore described and exemplified.	105
50	an anaerobic vessel next to Petri dishes	-	
	containing anaerobic cultures. 20 ml. water	VENNER, SHIPLEY & CO.,	
	are introduced with a syringe into the paper	Chartered Patent Agents.	
	sachet and the vessel is closed and left to	Rugby Chambers,	
	incubate. After only a few minutes, a	2, Rugby Street,	110
55	practically oxygen-free atmosphere is pro-	London, WC1N 3QU.	0
	duced in the anaerobic vessel.	Agents for the Applicants.	